

WHAT IS CLAIMED IS:

1. A method of constructing a contig containing the complete sequence of a piece of DNA comprising:

subcloning fragments of said piece of DNA;

5 obtaining the complete sequence of a portion of said subcloned fragments;

obtaining the sequence of the ends of the remainder of said subcloned fragments;

identifying overlapping subclones to construct one or more scaffolds covering the complete sequence of said piece of DNA; and

10 completely sequencing said overlapping subclones.

2. The method of Claim 1, wherein said subclones represent about 10 insert equivalents of said piece of DNA.

3. The method of Claim 2, wherein said step of obtaining the complete sequence of a portion of said subcloned fragments comprises obtaining the complete
15 sequence of about 10 percent of the subcloned fragments.

4. The method of Claim 1, wherein said overlapping set of subclones comprises about 1 to about 5 insert equivalents.

5. The method of Claim 4, wherein said overlapping set of subclones comprises about 2.5 insert equivalents.

20 6. The method of Claim 1, wherein said step of subcloning fragments of said piece of DNA comprises subcloning fragments of said piece of DNA into a vector comprising a high copy number origin of replication having at least one cloning site therein, a low copy number origin of replication, at least one copy number indicator for indicating the copy number of said vector in cells, and a vector maintenance marker for
25 selecting cells containing said vector, wherein said at least one cloning site is positioned in said high copy number origin of replication such that the ability of said high copy number origin of replication to direct replication is not disrupted when no insert has been cloned into said at least one cloning site and is disrupted when an insert is cloned into said at least one cloning site.

30 7. A method of generating a deletion in an insert comprising:

obtaining a linear piece of vector DNA comprising an origin of replication, a deletion indicator, an insert in which it is desired to make deletions, a deletion indicator, and a vector maintenance marker;

5 generating deleted vectors by contacting said linear piece of vector DNA with a solution consisting essentially of mung bean nuclease;
circularizing said deleted vectors;
introducing said circularized deleted vectors into cells; and
identifying cells carrying deleted vectors in which a portion of said of said insert is deleted and said origin of replication is not deleted.

10 8. The method of Claim 7, wherein said origin of replication is at a first end of said linear piece of vector DNA, said deletion indicator is at a second end of said linear piece of vector DNA, said insert is adjacent to said deletion indicator, and said vector maintenance marker is adjacent to said origin of replication.

15 9. The method of Claim 8, wherein said origin of replication comprises a low copy number origin of replication.

10. A method for identifying vectors containing a DNA insert, comprising the steps of:

20 subjecting a plurality of vectors to insertion conditions under which an insert is placed in at least some of said vectors, wherein said vectors have a first copy number if no insert occurs and a second copy number if said insert occurs; and

identifying at least some vectors containing an insert by screening said vectors for said second copy number.